

Table S1. WGS species predictions compared to line-probe assays for rarer species and mixtures

LPA result	WGS result	No. of isolates
<i>M. interjectum</i>	<i>M. intracellulare</i>	2
<i>M. interjectum</i>	<i>M. gordonaee</i>	1
<i>M. interjectum</i>	<i>M. szulgai</i>	1
<i>M. interjectum</i>	Failed	1
<i>M. scrofulaceum</i>	<i>M. brisbanense*</i>	1
<i>M. scrofulaceum</i>	<i>M. chimaera</i>	1
<i>M. scrofulaceum</i>	<i>M. chimaera; M. intracellulare</i>	1
<i>M. scrofulaceum</i>	<i>M. intracellulare</i>	1
<i>M. genevense</i>	<i>M. lentiflavum</i>	2
<i>M. goodii</i>	<i>M. smegmatis</i>	1
<i>M. goodii</i>	Failed	1
<i>M. lentiflavum</i>	<i>M. lentiflavum</i>	5
<i>M. mucogenicum</i>	<i>M. llatzerense*</i>	11
<i>M. mucogenicum</i>	<i>M. ratisbonense*</i>	5
<i>M. mucogenicum</i>	<i>M. tuberculosis; M. chimaera; M. intracellulare</i>	1
<i>M. mucogenicum</i>	Failed	3
<i>M. simiae</i>	<i>M. simiae</i>	2
<i>M. szulgai</i>	<i>M. szulgai</i>	4
<i>M. szulgai</i>	<i>M. angelicum*</i>	1
<i>M. szulgai</i>	Failed	1
Total rarer species		46
<i>M. tuberculosis; M. abscessus</i>	<i>M. tuberculosis</i>	1
<i>M. tuberculosis; M. avium</i>	<i>M. tuberculosis; M. avium</i>	1
<i>M. tuberculosis; M. avium</i>	<i>M. tuberculosis</i>	1
<i>M. tuberculosis; M. avium</i>	<i>M. avium</i>	1
<i>M. tuberculosis; M. chelonae</i>	<i>M. tuberculosis</i>	1
<i>M. tuberculosis; M. chelonae</i>	<i>M. chelonae</i>	1
<i>M. tuberculosis; M. intracellulare</i>	<i>M. tomidae*</i>	1
<i>M. tuberculosis complex; M. avium</i>	<i>M. avium</i>	1
<i>M. tuberculosis complex; M. intracellulare</i>	<i>M. tuberculosis</i>	1
<i>M. abscessus; M. avium</i>	<i>M. abscessus</i>	1
<i>M. abscessus; M. fortuitum</i>	<i>M. abscessus</i>	1
<i>M. abscessus; M. intracellulare</i>	<i>M. abscessus</i>	2
<i>M. avium; M. intracellulare</i>	<i>M. avium; M. intracellulare</i>	1
<i>M. avium; M. intracellulare</i>	<i>M. avium; M. chimaera</i>	1
<i>M. avium; M. intracellulare</i>	<i>M. avium</i>	1
<i>M. avium; M. intracellulare</i>	<i>M. chimaera</i>	1
<i>M. avium; M. xenopi</i>	<i>M. avium</i>	1
<i>M. intracellulare; M. gordonaee</i>	<i>M. chimaera; M. gordonaee</i>	1
<i>M. intracellulare; M. gordonaee</i>	<i>M. chimaera</i>	1
<i>M. intracellulare; M. gordonaee</i>	<i>M. gordonaee</i>	1
<i>M. intracellulare; M. xenopi</i>	<i>M. chimaera</i>	1
<i>M. intracellulare; M. xenopi</i>	Failed	1
<i>M. gordonaee; M. kansasii</i>	Failed	1
<i>M. gordonaee; M. xenopi</i>	<i>M. tuberculosis</i>	1
Total mixtures		25

* Organism not in Mycobacterium CM/AS catalogue

Table S2. Repeat species tests for discordant samples

	No. of samples	Original species		Repeat species	
		LPA	WGS	LPA	WGS
Now concordant - LPA changed (n=15)					
	1	<i>M. abscessus</i>	<i>M. avium</i>	<i>M. avium</i>	<i>M. avium</i>
	1	<i>M. avium</i>	<i>M. shimoidei</i>	<i>M. shimoidei</i>	<i>M. shimoidei</i>
	1	<i>M. chelonae</i>	<i>M. abscessus</i> [†]	<i>M. abscessus</i>	<i>M. abscessus</i>
	5	<i>M. fortuitum</i>	<i>M. peregrinum</i> [†]	<i>M. peregrinum</i>	<i>M. peregrinum</i>
	1	<i>M. gordonaie</i>	<i>M. avium</i>	<i>M. avium</i>	<i>M. avium</i>
	1	<i>M. kansasii</i>	<i>M. malmoense</i>	<i>M. malmoense</i>	<i>M. malmoense</i>
	1	<i>M. kansasii</i>	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>
	1	<i>M. tuberculosis</i>	<i>M. africanum</i> [†]	<i>M. africanum</i>	<i>M. africanum</i>
	1	<i>M. tuberculosis</i>	<i>M. africanum</i> [†]	<i>M. tuberculosis and M. africanum</i>	<i>M. africanum</i>
	1	<i>M. ulcerans</i>	<i>M. marinum</i>	<i>M. marinum</i>	<i>M. marinum</i>
	1	<i>M. xenopi</i>	<i>M. abscessus</i>	<i>M. abscessus</i>	<i>M. abscessus</i>
Now concordant - WGS changed (n=16)					
	1	<i>M. africanum</i>	<i>M. tuberculosis</i> [†]	<i>M. africanum</i>	<i>M. africanum</i>
	1	<i>M. avium</i>	<i>M. chelonae</i>	<i>M. avium</i>	<i>M. avium</i>
	4	<i>M. avium</i>	<i>M. tuberculosis</i>	<i>M. avium</i>	<i>M. avium</i>
	1	<i>M. bovis</i>	<i>M. bovis (BCG strain)</i> [†]	<i>M. bovis</i>	<i>M. bovis</i>
	1	<i>M. bovis</i>	<i>M. fortuitum</i>	<i>M. bovis</i>	<i>M. bovis</i>
	1	<i>M. chelonae</i>	<i>M. llatzerense</i> [*]	<i>M. chelonae</i>	<i>M. chelonae</i>
	1	<i>M. chelonae</i>	<i>M. tuberculosis</i>	<i>M. chelonae</i>	<i>M. chelonae</i>
	1	<i>M. intracellulare</i>	<i>M. gordonaie</i>	<i>M. intracellulare</i>	<i>M. intracellulare</i>
	1	<i>M. intracellulare</i>	<i>M. tuberculosis</i>	<i>M. intracellulare</i>	<i>M. chimaera</i>
	1	<i>M. intracellulare</i>	<i>M. tuberculosis and M. avium</i>	<i>M. intracellulare</i>	<i>M. chimaera</i>
	1	<i>M. malmoense</i>	<i>M. tuberculosis</i>	<i>M. malmoense</i>	<i>M. malmoense</i>
	1	<i>M. tuberculosis</i>	<i>M. africanum</i> [†]	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>

	1	<i>M. xenopi</i>	<i>M. tuberculosis and M. avium</i>	<i>M. xenopi</i>	<i>M. xenopi</i>
Now concordant - Both changed (n=1)					
	1	<i>M. intracellulare</i>	<i>M. tomidae*</i>	<i>M. intracellulare and M. avium</i>	<i>M. avium</i>
Still discordant - No change (n=22)					
	1	<i>M. chelonae</i>	<i>M. llatzerense*</i>	<i>M. chelonae</i>	<i>M. llatzerense*</i>
	1	<i>M. chelonae</i>	<i>M. ratisbonense*</i>	<i>M. chelonae</i>	<i>M. ratisbonense*</i>
	1	<i>M. fortuitum</i>	<i>M. farcinogenes*†</i>	<i>M. fortuitum</i>	<i>M. farcinogenes*†</i>
	3	<i>M. fortuitum</i>	<i>M. porcinum*†</i>	<i>M. fortuitum</i>	<i>M. porcinum*†</i>
	2	<i>M. fortuitum</i>	<i>M. septicum*†</i>	<i>M. fortuitum</i>	<i>M. septicum*†</i>
	1	<i>M. intracellulare</i>	<i>M. arosiense*†</i>	<i>M. intracellulare</i>	<i>M. arosiense*†</i>
	2	<i>M. intracellulare</i>	<i>M. colombiense*†</i>	<i>M. intracellulare</i>	<i>M. colombiense*†</i>
	2	<i>M. intracellulare</i>	<i>M. marseillense*†</i>	<i>M. intracellulare</i>	<i>M. marseillense*†</i>
	2	<i>M. intracellulare</i>	<i>M. paraffinicum</i>	<i>M. intracellulare</i>	<i>M. paraffinicum</i>
	6	<i>M. intracellulare</i>	<i>M. tomidae*</i>	<i>M. intracellulare</i>	<i>M. tomidae*</i>
	1	<i>M. intracellulare</i>	<i>M. triplex*</i>	<i>M. intracellulare</i>	<i>M. triplex*</i>
Still discordant - LPA changed (n=2)					
	2	<i>M. peregrinum</i>	<i>M. farcinogenes*†</i>	<i>M. fortuitum</i>	<i>M. farcinogenes*†</i>
Still discordant - WGS changed (n=6)					
	1	<i>M. fortuitum</i>	<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. porcinum*†</i>
	1	<i>M. fortuitum</i>	<i>M. porcinum*†</i>	<i>M. fortuitum</i>	<i>M. tuberculosis</i>
	1	<i>M. fortuitum</i>	<i>M. septicum*†</i>	<i>M. fortuitum</i>	<i>M. avium</i>
	1	<i>M. fortuitum</i>	<i>M. septicum*†</i>	<i>M. fortuitum</i>	<i>M. tuberculosis</i>
	1	<i>M. intracellulare</i>	<i>M. tuberculosis</i>	<i>M. intracellulare</i>	<i>M. tomidae*</i>
	1	<i>M. peregrinum</i>	<i>M. kansasii</i>	<i>M. peregrinum</i>	<i>M. farcinogenes*†</i>
Failed (n=15)					
	1	<i>M. abscessus/M. chelonae</i>	<i>M. llatzerense*</i>	Failed	Failed
	2	<i>M. chelonae</i>	<i>M. abscessus†</i>	Failed	Failed
	1	<i>M. fortuitum</i>	<i>M. gordonaee</i>	Failed	Failed
	1	<i>M. fortuitum</i>	<i>M. septicum*†</i>	<i>M. fortuitum</i>	Failed
	1	<i>M. gordonaee</i>	<i>M. intracellulare</i>	Failed	Failed

	1	<i>M. gordonaе</i>	<i>M. tuberculosis</i>	Failed	Failed
	1	<i>M. intracellulare</i>	<i>M. abscessus</i>	Failed	Failed
	1	<i>M. intracellulare</i>	<i>M. avium</i> †	<i>M. avium</i>	Failed
	1	<i>M. intracellulare</i>	<i>M. paraffinicum</i>	<i>M. intracellulare</i>	Failed
	1	<i>M. intracellulare</i>	<i>M. tomidae</i> *	<i>M. intracellulare</i>	Failed
	1	<i>M. intracellulare</i>	<i>M. tomidae</i> *	Failed	Failed
	1	<i>M. malmoense</i>	<i>M. chimaera</i>	Failed	<i>M. chimaera</i>
	1	<i>M. malmoense</i>	<i>M. intracellulare</i>	Failed	<i>M. intracellulare</i>
	1	<i>M. tuberculosis</i>	<i>M. africanum</i> †	Failed	<i>M. avium</i>

* Organism not in Mycobacterium CM/AS catalogue

† Organism is in same complex as LPA species

Notes:

The *M. tuberculosis* complex includes: *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* (BCG strain)

The *M. abcessus* complex includes: *M. abcessus*, *M. chelonae*

The *M. avium* complex includes: *M. avium*, *M. intracellulare*, *M. chimaera*, *M. arosiense*, *M. colombiense*, *M. marseillense*

The *M. fortuitum* complex includes: *M. fortuitum*, *M. mageritense*, *M. peregrinum*, *M. septicum*, *M. porcinum*, *M. farcinogenes*

Table S3. Repeat WGS in-silico LPA predictions compared to MTBDRplus, for discordant samples. MUT = Mutation, LWT = Loss of wild type, WT = Wild type, F = Failed retesting.

	No. of samples	Gene	Original result		Repeat result	
			MTBDRplus	WGS	MTBDRplus	WGS
Now concordant - MTBDRplus changed (n=4)						
	1	<i>inhA</i>	LWT	WT	WT	WT
	2	<i>katG</i>	WT	MUT	MUT	MUT
	1	<i>rpoB</i>	WT	LWT	LWT	LWT
Still discordant - No change (n=3)						
	1	<i>rpoB</i>	MUT	LWT	MUT	LWT
	2	<i>rpoB</i>	WT	LWT	WT	LWT
Still discordant - MTBDRplus changed (n=1)						
	1	<i>rpoB</i>	WT	LWT	MUT	LWT
Failed (n=5)						
	1	<i>inhA</i>	MUT	WT	F	F
	1	<i>katG</i>	WT	MUT	F	F
	2	<i>rpoB</i>	WT	LWT	F	F
	1	<i>rpoB</i>	WT	LWT	F	LWT

Table S4. LPA predictions compared to phenotypic DST. MUT = Mutation, LWT = Loss of wild type, WT = Wild type, F = Failed to produce a clear result. Only includes samples where both a phenotypic and LPA result could be found.

	Phenotypically Resistant				Phenotypically Susceptible				Excluding failed (95% CI)		Overall concordance (95% CI)			
	LPA prediction				Total	LPA prediction				Total	Sensitivity*	Specificity*	Excluding failed	Including failed†
	MUT	LWT	WT	F		MUT	LWT	WT	F					
Isoniazid	63	0	10	1	74	0	0	618	0	618	86.3 (76.2-93.2)	100.0 (99.4-100.0)	98.6 (97.4-99.3)	98.4 (97.2-99.2)
Rifampicin	19	2	6	0	27	0	0	664	0	664	80.0 (57.7-91.4)	100.0 (99.4-100.0)	98.8 (97.7-99.5)	98.8 (97.7-99.5)
All	82	2	16	1	101	0	0	1282	0	1282	84.0 (75.3-90.6)	100.0 (99.7-100)	98.8 (98.1-99.3)	98.8 (98.0-99.3)

* For sensitivity and specificity calculations, all LWT LPA mutations were treated as Resistant, as this would usually be the clinical interpretation. MTBDRplus does not claim to predict susceptibility, (only absence of known resistance mutations), but we have treated WT calls as Susceptible in order to aid comparison.

† i.e. treating all failed results as discordant